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Evaluation of a Pelleted Bait Containing Methyl Anthranilate as a Bird Repellent

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Abstract: No-till agriculture involves the use of granular pesticide formulations, chemically treated seeds, and pelleted baits. Some of these may accidentally kill birds. We have tested whether methyl anthranilate (MA), a known bird repellent, would eliminate consumption of a pelleted bait. In two laboratory experiments and an outdoor aviary trial, cowbirds (*Molothrus ater* Bodd.) were presented with pellets containing pesticide and MA, pellets containing pesticide but no MA, and carrier pellets without pesticide or MA. Consumption of any formulation was low, but the addition of MA significantly decreased bait loss in the laboratory, and prevented the disappearance of bait in the outdoor trial.

1 INTRODUCTION

No-tillage conservation farming is becoming common for both environmental and economic reasons.[1] Overall, these farming practices benefit wildlife because they preserve food and cover, and because they often do not involve broadcast spray applications of pesticide.[2] However, granular pesticides, pelleted baits, and chemically treated seeds (hereafter referred to collectively as PF) are essential components of no-tillage farming; some of these products are toxic to birds[3–5] and may easily be mistaken for food or grit.[6]

Because of the dangers to wildlife, the Environmental Protection Agency restricts and occasionally bans the use of various PF. There is ample statutory justification for this position. The Migratory Bird Treaty Act sets zero tolerance for bird mortality from human activities.[7]

One solution to the problem of accidental poisoning by PF may be the use of bird-repellent additives in these products. Methyl anthranilate (MA) could be one such additive. This human food flavoring is offensive to many bird species at concentrations between 5 and 10 g kg^{-1},[8,9] and the available data suggest that it is as effective in the field as it is in the laboratory.[10,11] It is not considered toxic to mammals or birds, available toxicity values by dietary and acute oral exposure being: mouse $LC_{50} = 3900$ mg kg^{-1}; rat $LD_{50} = 3288$ mg kg^{-1}; quail $LC_{50} > 5600$ mg kg^{-1}; quail $LD_{50} > 290$ mg kg^{-1} (precise avian LD_{50} values have not been determined—birds regurgitate higher dosages) (P. Vogt, pers. comm.).

In the present assessment, MA was incorporated into PF pellets and the pellets were presented to cowbirds in two laboratory experiments and an outdoor aviary evaluation. Our primary objective was to determine whether MA would decrease PF ingestion. In addition, because pellets containing pesticide but no MA, and pellets without pesticide or MA also were presented, we were able to examine whether the pelleted baits, *per se*, would be ingested by cowbirds.

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2 EXPERIMENTAL METHODS

2.1 Birds

Adult brown-headed cowbirds (*Molothrus ater* Bodd.) were decoy-trapped in July 1991 and April 1992 in Sandusky, Ohio, and air-shipped to Philadelphia, Pennsylvania. This bird was selected for use because it is potentially at risk from PF formulations. For laboratory experiments (July–August 1991), birds were transported from the airport to the Monell Chemical Senses Center. For aviary tests (April 1992), birds were transported to the Rutgers Agricultural Experiment Station, Bridgeton, New Jersey.

2.2 Chemicals

Six pelleted formulations were provided by the Agricultural Research Division of American Cyanamid Corporation, Princeton, NJ. Two of the formulations (B1M1, M1M1) contained edible bait, an experimental molluscicide (proprietary, 10 g kg^{-1}, hereafter referred to as pesticide) and methyl anthranilate (MA, CAS # 134-20-3, 10 g kg^{-1}), two (B1M0, M1M0) contained edible bait and the experimental pesticide (10 g kg^{-1}), and two (B0M0, M0M0) contained edible bait only. The sole difference between the two formulations in each pair was a difference in the inert carrier base. The three formulations used in the outdoor aviary trial (B1M1, B1M0, B0M0) were selected on the basis of several formulation criteria, one of which was superior environmental durability.

2.3 Laboratory experiments

Upon arrival at Monell, the birds were weighed, and then individually caged (61 × 36 × 41 cm) under a 12:12 h light:dark cycle. During a two-week adaptation period prior to testing, all birds were given free access to Purina Flight Bird Conditioner (feed; Purina Mills, St. Louis, Mo.), and water. The feed was presented on top of 500 ml of sterilized sand in plastic tubs (30 × 15 × 15 cm). Sand particles were uniformly smaller than US standard sieve size 18 in all phases of Experiments 1 and 2.

Throughout both experiments, birds were deprived of food overnight to encourage feed and pellet consumption during testing. During the four-day pretreatment period preceding the first experiment, all birds were given 30 g of feed and 1 g of control (M0M0) pellets in plastic tubs containing 500 ml of sand (described above) during a 3-h measurement period. While the feed was presented in a metal cup (7 cm diam.) positioned in the center of the tub, the pellets were evenly spread over the sand surface. This separation was dictated by practical considerations (see below).

The feed remaining in each metal cup at the end of each session was weighed, and differences from the beginning of the trial were defined operationally as consumption. To evaluate pellet ingestion, the following method was adopted. First, pellet size was standardized prior to each trial as particles that passed through a # 16 sieve, but were caught on a # 18 sieve. This pellet size range encompassed most pellets, but also was the same size as feed granules; hence separate presentation.

At the end of each session, tubs of sand were poured through the # 18 sieve, and pellets trapped on the sieve were weighed. Visual inspection of sieved sand indicated that this procedure recovered all of the pellets (which were easily seen because of their light blue color). In addition, when feces with pellets on them were found in tubs at the conclusion of a trial, the pellets were separated from the feces, collected, and left at room temperature for one hour before being returned to the sieve-collected sample for weighing. To control for moisture absorption or loss by the pellets, 1-g samples were left in the testing room during each session. Mean percentage changes in the weight of these samples were used to adjust the weight of pellet samples recovered from tubs. Because moisture-associated changes in feed weight were negligible in pilot tests, no similar controls for moisture absorption or loss were implemented.

At the end of the pretreatment period, all birds were weighed and ranked on the basis of consumption. These rankings were used to assign birds to six groups (n = 6/group) that were balanced with respect to feed intake. Pellet consumption was not used to assign birds to groups because no measurable ingestion occurred.

On each of four treatment days, each of the six groups was given 30 g of feed and 1 g of pellets for 3 h, as described above. Groups 1 and 2 were presented with M1M1 or B1M1 pellets. Groups 3 and 4 were given M1M0 or B1M0 pellets. Groups 5 and 6 were given M0M0 or B0M0 pellets. At the end of the treatment period, birds were re-weighed.

The procedures followed in Experiment 2 were essentially identical to those just described, except that both feed and pellets were presented on the sand surface. The same birds, assigned to the same treatment groups, were used in both experiments. No attempt was made to measure feed and pellet consumption because, as indicated above, the feed and the pellets could not be separated readily. Instead, mortalities and morbidity were recorded.

Three-factor analyses of variance (ANOVA) were used to assess pellet and feed consumption in the first experiment. The factors in these analyses were treatment group, period, and day. In addition, a two-factor (treatment groups, days) ANOVA was used to examine changes in body weights during the treatment period. In all cases, Tukey tests[12] were used to isolate significant differences among means. With the exception of body weight values, data from the second experiment were not statistically evaluated because no birds became visibly sick, and none died. As in Experiment 1, body weights were evaluated in a two-factor (treatment group, days) ANOVA. Data were the weights of birds at the conclusion of Experiment 1 and their weights at the end of Experiment 2.

2.4 Outdoor aviary tests

Upon arrival at the Experiment Station, the birds were weighed, individually banded, and then released into 16 large ($6 \times 3 \times 3$ m) rectangular enclosures ($n = 12$ birds/enclosure). The ground beneath the enclosures had been tilled. During a one-week pretreatment period prior to testing, and throughout the remainder of the trial, all birds were given free access to sunflower seeds, cracked corn and wheat seeds that were liberally spread on the ground within the enclosures. Water was freely available from two large (30 cm diam., 12 cm deep) plastic bowls, one placed at each end of each enclosure. Mortalities were recorded daily at 0630 and 1700 h, and dead birds were removed. Maximum and minimum soil and air temperatures, wind speeds, total rainfall, rain intensity (cm h^{-1}), and percentage humidity were recorded daily throughout the test by the Rutgers Agricultural Experiment Station.

On the day following the last day of pretreatment, 12 enclosures were randomly assigned to three treatments ($n = 4$ enclosures/treatment). The treatments were: B1M1 (i.e. pellets containing MA and pesticide); B1M0 (i.e. pellets containing pesticide only), and B0M0 (i.e. pellets containing edible bait only). In all cases, the pellet application rate was 5·5 kg ha^{-1}, and pellets were spread evenly throughout the enclosures. Three of the remaining enclosures were assigned to a control condition in which no pellets were presented. The last enclosure was discarded, because most of the birds (9/12) escaped following an avian predator attack. Finally, in another enclosure without birds (hereafter referred to as E-17), B0M0 pellets were spread on the ground so that particle weathering in the absence of birds could be examined.

After treatment applications were completed, three 929-cm^2 sampling plots were arbitrarily selected within each B1M1, B1M0, and B0M0 enclosure, and the number of pellets within each plot was counted. In addition, in E-17, six 929-cm^2 sampling plots were selected and outlined with orange fluorescent paint; the number of pellets within each plot was assessed.

On each of the next 14 (treatment period) days, mortalities were recorded within each enclosure at 0630 and 1700 h. Also, the numbers of pellets in E-17 sampling plots were counted. Pellets were not counted daily in the other enclosures because measurement would have involved entering the enclosures and disturbing the soil surface.

On treatment day 15, the 12 treated enclosures were re-entered, and three 929-cm^2 sampling plots were selected arbitrarily within each. The number of pellets per plot was recorded, as were the number of pellets in each E-17 sampling plot. All birds in all 15 enclosures were then netted, and weighed. With the exception of 20 control birds that were transported to the Monell Center for additional experiments, all birds were killed by cervical dislocation. Digestive tracts were removed immediately, and frozen by immersion in liquid nitrogen. After freezing, the tracts and carcasses were packed on dry ice for transport to the Monell Center. At Monell, all samples were stored in an ultra-deep freeze (−40°C) until they could be shipped to American Cyanamid for pesticide residue analyses.

Two-factor ANOVAs were used to evaluate body weight and pellet counts. The factors in these analyses were treatment and days. Control group enclosures were not included as a level of the treatment factor in the analysis of pellet counts because no pellets had been spread in them. In addition, E-17 pellet counts were evaluated in a one-factor repeated measures ANOVA. In all cases, Tukey post-hoc tests were used to isolate significant differences among means. Mortalities were not statistically evaluated because few birds died. During pretreatment, there were nine deaths (two in each of three enclosures, and one in each of another three enclosures). During the post-treatment period, there were only three deaths overall, and these occurred in B0M0 enclosures. All 12 deaths resulted from decapitation, and were attributed to predator attacks.

3 RESULTS

3.1 Laboratory

3.1.1 Experiment 1: Pellet consumption

Overall, consumption was very low (Fig. 1, top). However, there were significant differences between periods ($F = 5{\cdot}48$; 1, 30 df; $P < 0{\cdot}026$). Mean consumption was significantly greater during the treatment period than during pretreatment. There was also a group × period interaction ($F = 4{\cdot}51$; 5, 30 df; $P < 0{\cdot}003$). Post-hoc tests showed that consumption of pellets containing pesticide only (i.e. M1M0, B1M0) was significantly greater than that of control pellets (i.e. M0M0, B0M0) or pellets containing pesticide and MA (i.e. B1M1, M1M1). There were no other significant effects ($P > 0{\cdot}20$).

3.1.2 Experiment 1: Feed consumption

There were no significant differences among groups or periods in consumption ($P > 0{\cdot}25$; Fig. 1, middle). There also were no significant differences among days ($P > 0{\cdot}20$).

3.1.3 Experiment 1: Body weight

Overall, the mean weight of birds decreased significantly during the experiment ($F = 49{\cdot}5$; 1, 30 df; $P < 0{\cdot}0001$;

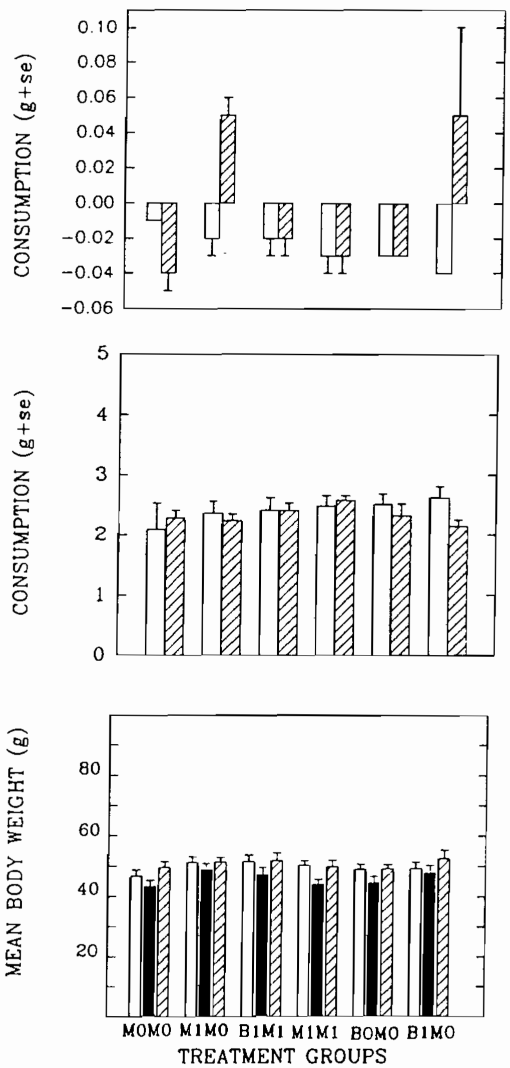

Fig. 1. (Top) Consumption of pellets by birds in all formulation groups: (▨) treatment; (□) pretreatment. Values less than 0 probably reflect increases in weight caused by moisture absorption. (Middle) Consumption of feed by birds in (▨) all treatment groups; (□) pretreatment. (Bottom) Mean body weights of birds in all treatment groups (□) prior to Experiment 1, (■) after Experiment 1, and (▨) following Experiment 2. Capped vertical bars represent standard errors of the means. These bars are absent in some cases because standard errors were extremely small. Groups: M0M0 = control pellets; M1M0 = pesticide only pellets; B1M1 = pesticide and MA pellets; M1M1 = pesticide and MA pellets; B0M0 = control pellets; B1M0 = pesticide only pellets.

Fig. 1, bottom). There were no significant differences among groups and no significant interaction between groups and days ($P > 0{\cdot}25$).

3.1.4 Experiment 2

There were no significant changes in body weight ($P > 0{\cdot}25$, Fig. 1, bottom).

3.2 Outdoor aviary trial

3.2.1 Weather

There was 2·26 cm of rain during the post-treatment period. Soil and air temperatures ranged from 0·6 to 16·8°C and −0·6 to 26·3°C, respectively. Wind speeds ranged from 0 to 39 km h^{-1} (Fig. 2).

3.2.2 Pellet counts

There were significant differences among treatment groups ($F = 10{\cdot}2$; 2, 9 df; $P < 0{\cdot}005$); pellet counts were significantly lower for B1M0 and B0M0 enclosures than for B1M1 enclosures (Fig. 3). Also, there was a significant difference between treatment days 1 and 15 ($F = 127{\cdot}8$; 1, 9 df; $P < 0{\cdot}00001$); overall, the number of pellets decreased. Finally, there was an interaction between treatment groups and days ($F = 23{\cdot}3$; 2, 9 df; $P < 0{\cdot}0005$). Post-hoc tests showed that while pellet counts decreased in B1M0 and B0M0 enclosures, there was no appreciable change in the number of pellets counted in B1M1 enclosures. There were no significant differences among groups on treatment day 1 ($P > 0{\cdot}25$).

When pellet counts in E-17 were examined, there was a significant days effect ($F = 8{\cdot}5$; 14, 70 df; $P < 0{\cdot}00001$). Post-hoc tests showed that there were more pellets in this enclosure on treatment day 1 than there were on any other day (Fig. 4). The mean pellet count for day 2 was significantly higher than mean counts for days 3, 4, 5, 6, 14, and 15. Increases in mean pellet counts and variance around these means from treatment day 7 through treatment day 13 were concurrent with periods of rainfall during that period (Fig. 2). Although not statistically comparable, E-17 pellet counts were numerically less than pellet counts in B1M1 enclosures but greater than those in B0M0 and B1M0 enclosures.

3.2.3 Body weights

There were significant differences between pretreatment day 1 and treatment day 15 ($F = 64{\cdot}2$; 1, 11 df; $P < 0{\cdot}00001$). Post-hoc evaluation showed that mean body weights increased for all treatment groups (Fig. 5). Otherwise, there were no significant effects.

4 DISCUSSION

The present experiments suggest two conclusions. First, the laboratory experiments showed that consumption of the pellet formulations was very low, even when the birds were deprived of food. Likewise, in the outdoor enclosures, no birds died or became sick after two weeks of exposure to pelleted baits, even when some ingestion or other exposure may have occurred (i.e. bait disappearance in B1M0 and B0M0 enclosures relative to E-17). Second, the laboratory data suggest that pellets containing pesticide only were more likely to be ingested

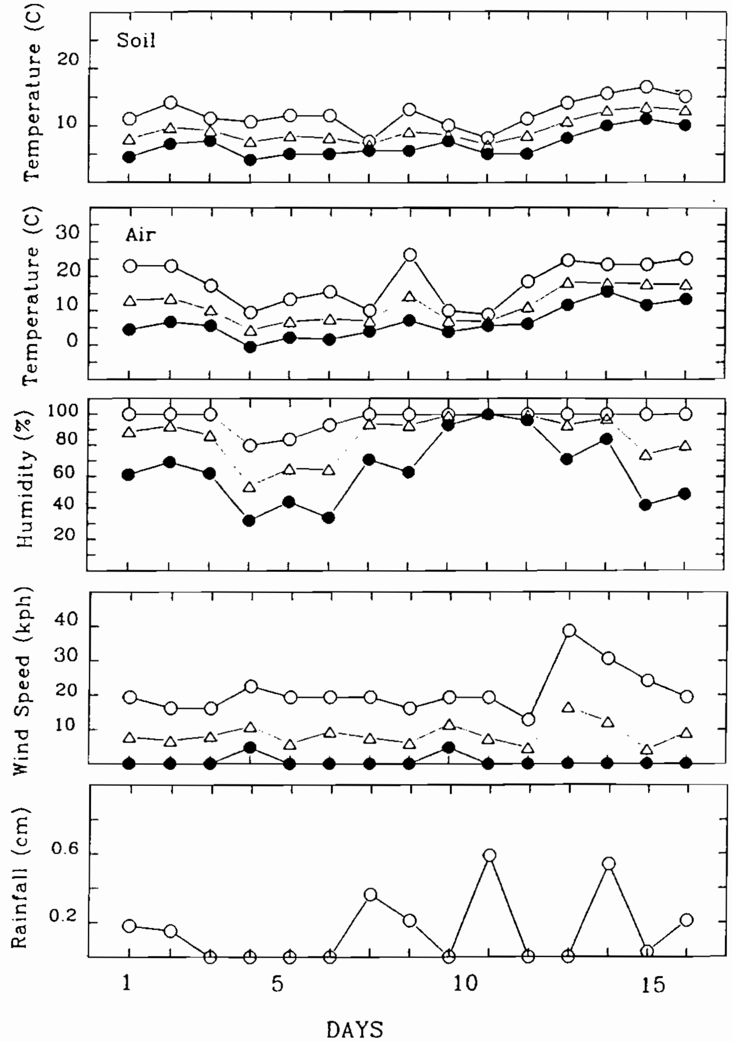

Fig. 2. Weather conditions during the treatment period in outdoor aviary tests. (○) Maximum, (●) minimum, (△) mean.

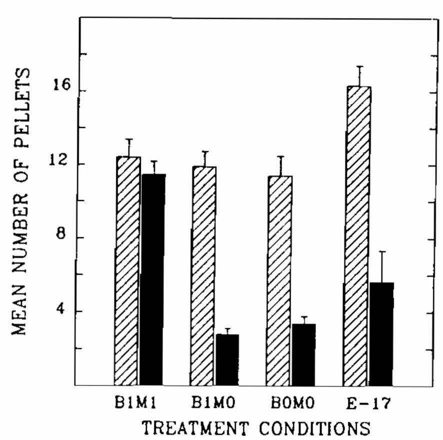

Fig. 3. Mean numbers of pellets counted in sampling plots within enclosures on treatment days (▨) 1 and (■) 15. Capped vertical bars represent standard errors of the means.

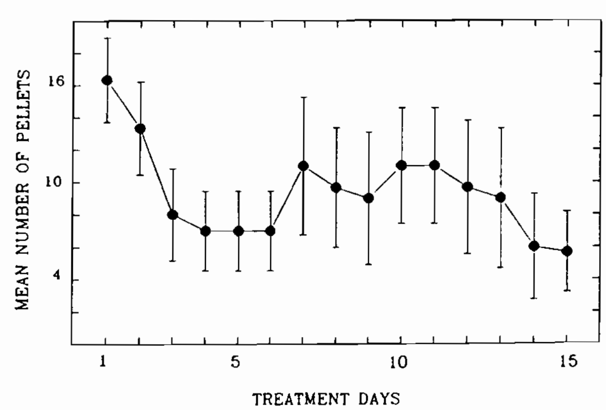

Fig. 4. Mean numbers of pellets counted in sampling plots within Enclosure 17. Capped vertical bars represent standard errors of the means.

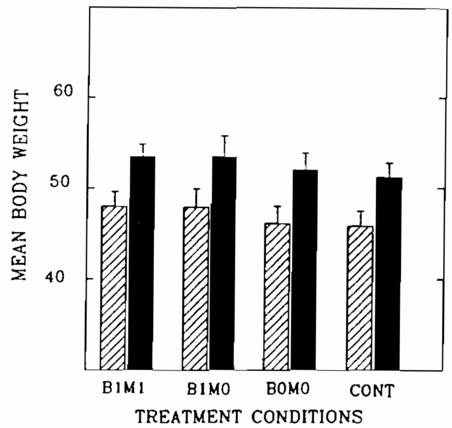

Fig. 5. Mean body weights on (▨) pretreatment day 1 and (■) treatment day 15. Capped vertical bars represent standard errors of the means.

(where ingestion was operationally defined as pellet weight decrease) than either control pellets or pellets containing pesticide and MA. This result could indicate that the pesticide enhanced consumption, although this inference is premature and quite probably reflects error variance, since consumption in all cases was so low. Nevertheless, the observation that pellets containing MA were relatively less likely to be eaten in the laboratory is consistent with the results of the subsequent aviary trial, i.e. pellets containing MA were less likely to disappear from the soil surface than were pellets without MA. Although the addition of MA to a PF could make it more resistant to weathering, the most likely explanation is that MA acted as an effective repellent, and birds avoided the repellent-adulterated pellets.

The apparent difference between pellet counts in E-17 and B1M1 enclosures on treatment day 15 is puzzling. Perhaps the difference reflects the number of sampling plots employed (six in E-17, three in each of the B1M1 enclosures). Alternatively, the activity of birds in B1M1 enclosures may have kept pellets exposed. This explanation is consistent with increases in E-17 pellet counts following rainfall that also disturbed the ground within the enclosure (Fig. 2).

While the findings are encouraging, they require cautious interpretation. First, the data are undoubtedly specific to the physical characteristics of the pelleted product tested, and influenced by the parameters of our experimental designs. For example, in the laboratory, sand was used as the substrate in both experiments, and birds may not have ingested PF because the sand served as an adequate source of grit. Likewise, birds may not have ingested much PF in the outdoor aviary trial because they had ready access to soil for grit, and an excess food supply. In any future laboratory work, we suggest that the substrate should not be a material that could serve as grit. In outdoor aviary trials, we suggest that birds be moderately deprived of food.

5 CONCLUSIONS

To reduce significantly or eliminate the hazards of PF to wildlife, a number of redundant features should be considered in the design of a product. These features include the use of colors that make PF indistinct from the substrate, a soft texture that diminishes the likelihood that particles will be ingested as grit, and the inclusion of a chemical repellent that is aversive to both birds and rodents (e.g. MA, *o*-aminoacetophenone,[13] pulegone[14]). The present experiments suggest that the inclusion of MA as a chemical repellent can reduce or eliminate ingestion of a pelleted bait by birds. To establish more solidly this conclusion, we recommend field tests with free-ranging birds.

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